FIGURE 1

Western Blot of Flk-1/SEAPS Immunoprecipitation with MAb DC101

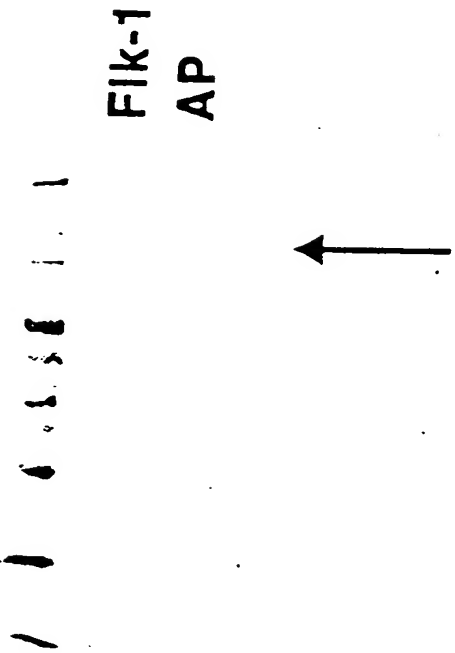


FIGURE 2A

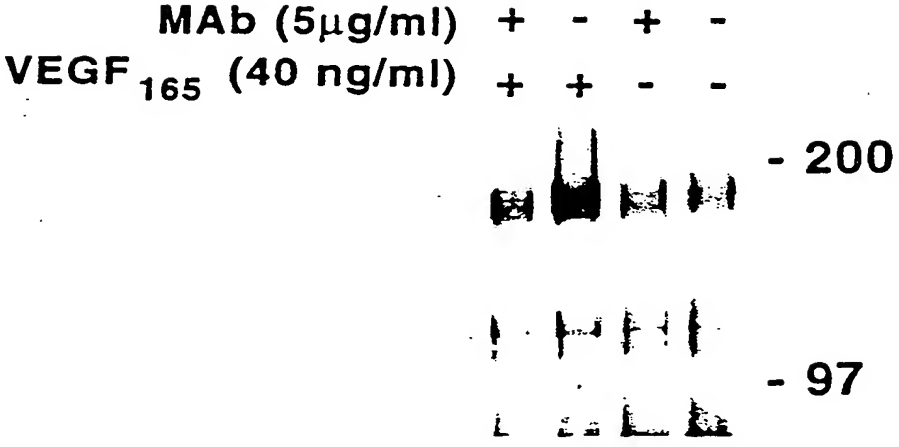


FIGURE 2B

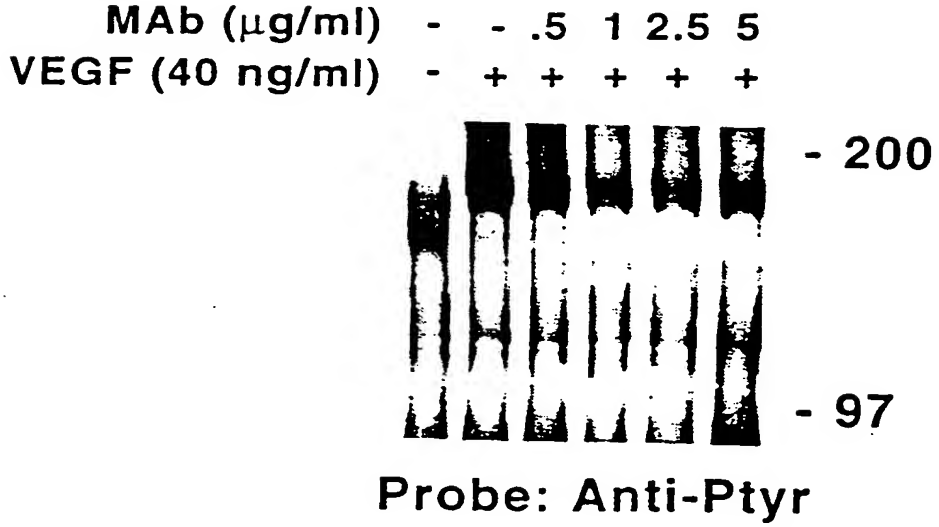


FIGURE 3A

FIGURE 3B

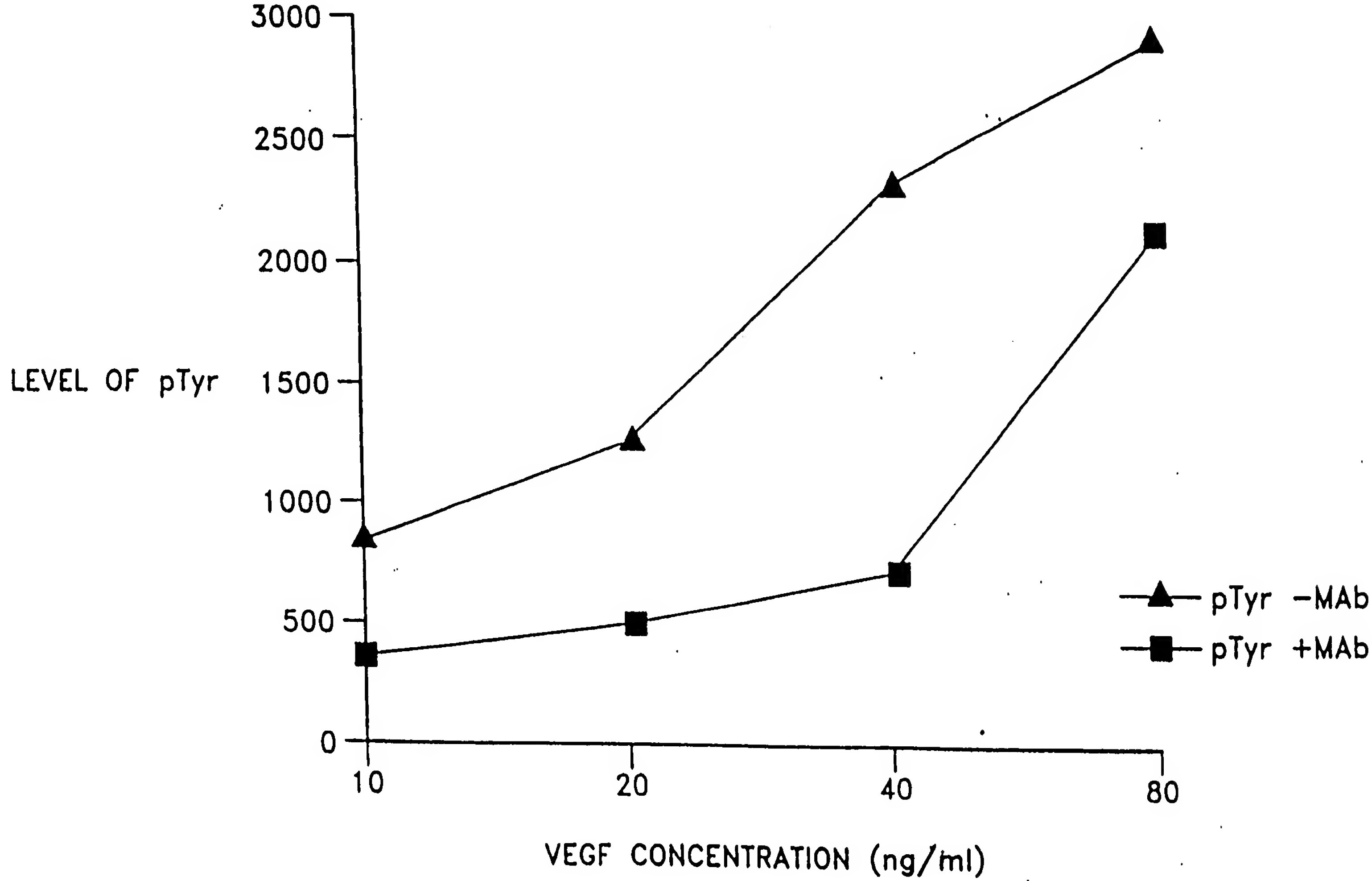
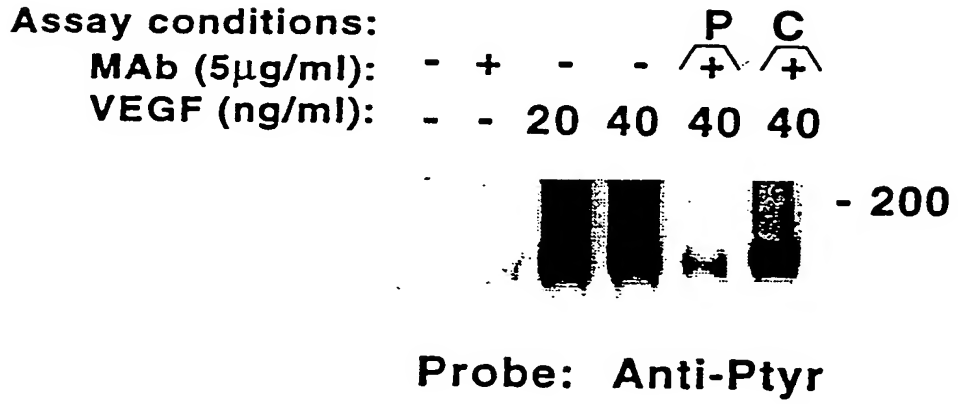

3000
2500
2000
1500
1000
500
0
LEVEL OF pTyr
10
20
40
80
VEGF CONCENTRATION (ng/ml)
pTyr −MAb
pTyr +MAb

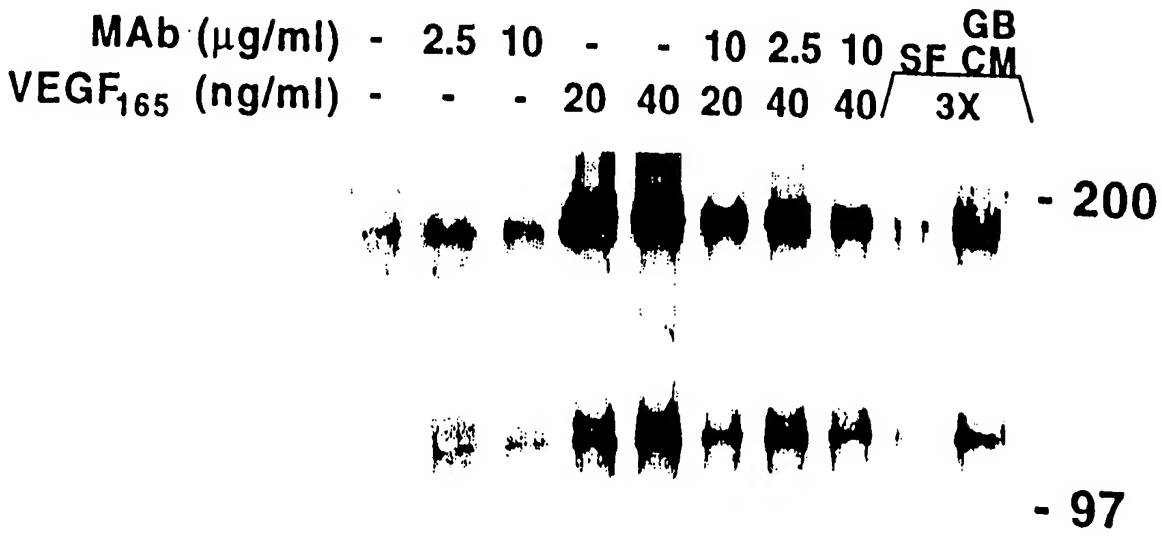
FIGURE 4

Inhibition of VEGF-Flk-1/fms activation by pre-bound MAb DC101



Assay conditions: P: MAb pre-bound 15'; VEGF 15'
C: Competitive assay; MAb + VEGF 15'

FIGURE 5



Probe: Anti-Ptyr

FIGURE 6 FACS ANALYSIS OF Anti-flk-1 MAb BINDING TO flk-1/fms TRANSFECTED 3T3 CELLS (C441)

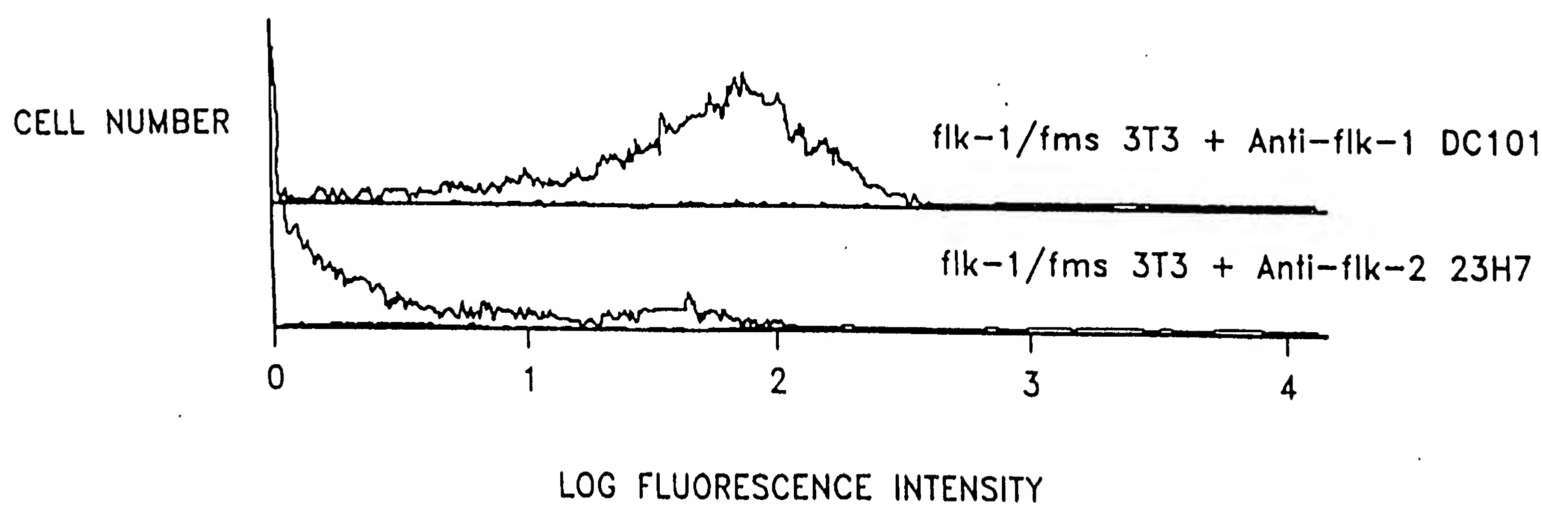


FIGURE 7 SATURATION BINDING OF MAb DC101 TO THE flk-1/fms RECEPTOR ON THE TRANSFECTED 3T3 CELL LINE C441

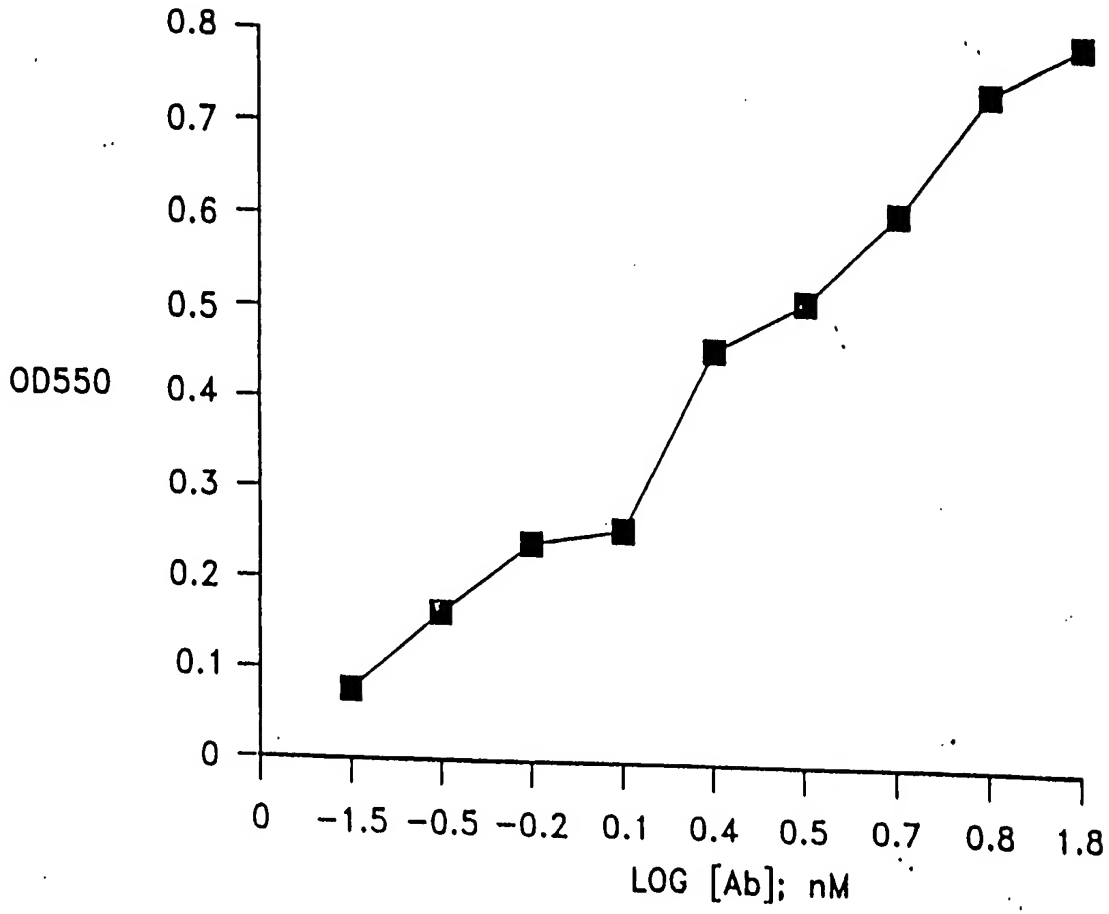


FIGURE 8

Immunoprecipitation of phosphorylated Flk-1/fms from VEGF stimulated Flk-1/fms transfected 3T3 cells

Antibodies:
1) Rat anti-flk-2 IgG_{2a} 2A13
2) Rat anti-flk-1 IgG_1 DC101
3) Rat anti-flk-2 IgG_1 23H7
4) Rabbit anti-fms polyclonal IM 133

FIGURE 9

Sensitivity of VEGF induced phosphorylation of the Flk-1/fms receptor to inhibition by MAb DC101

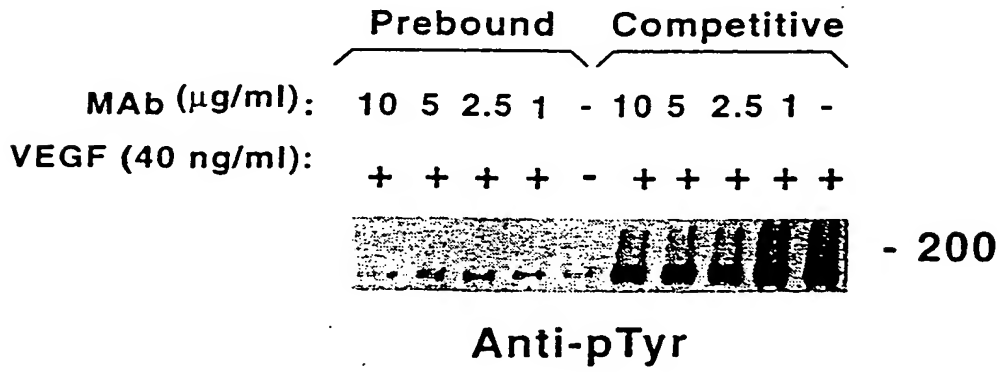
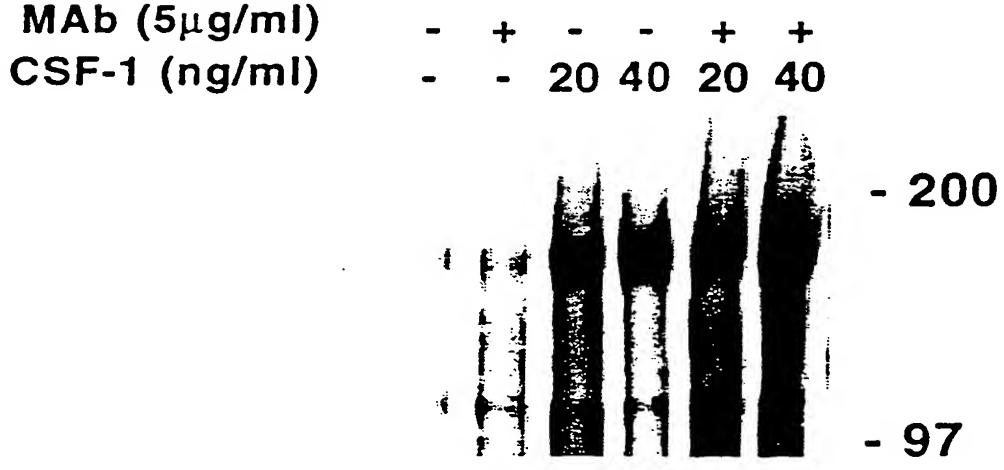


FIGURE 10

Effect of MAb DC101 on CSF-1 induced phosphorylation of the fms receptor



Probe: Anti-Ptyr

FIGURE 11

Specificity of MAb DC101 neutralization of the activated Flk-1/fms receptor

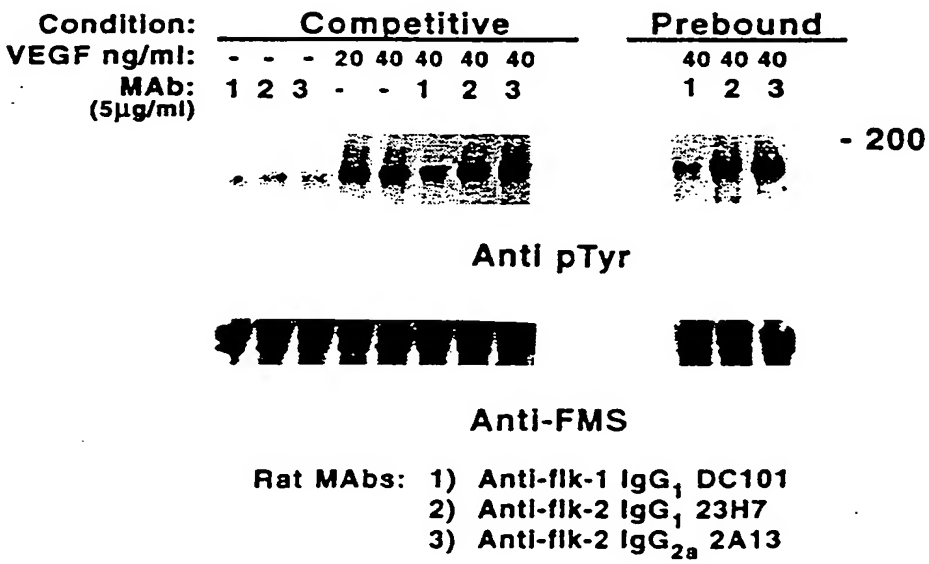


FIGURE 12

Immunoprecipitation of phosphorylated receptor bands from VEGF stimulated HUVEC cells

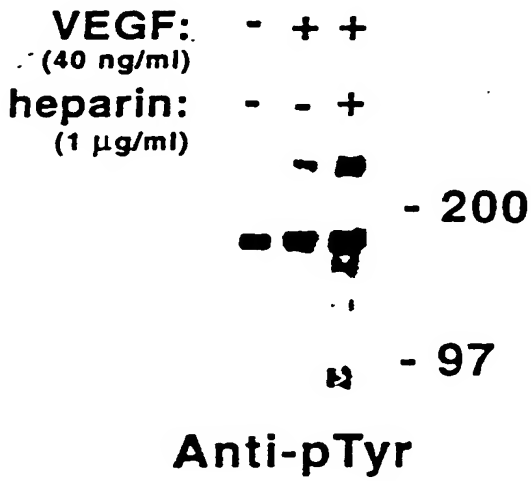


FIGURE 13

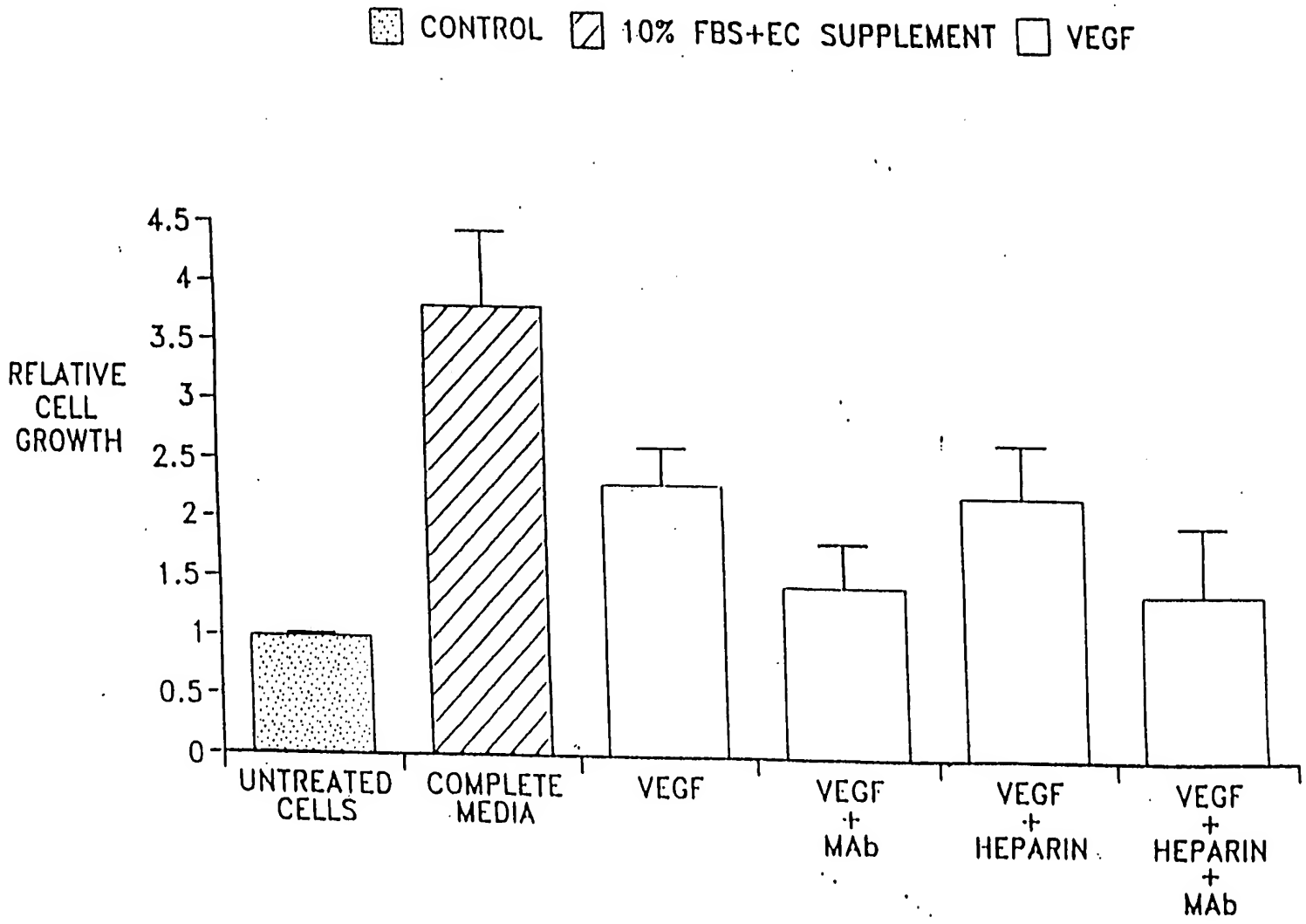
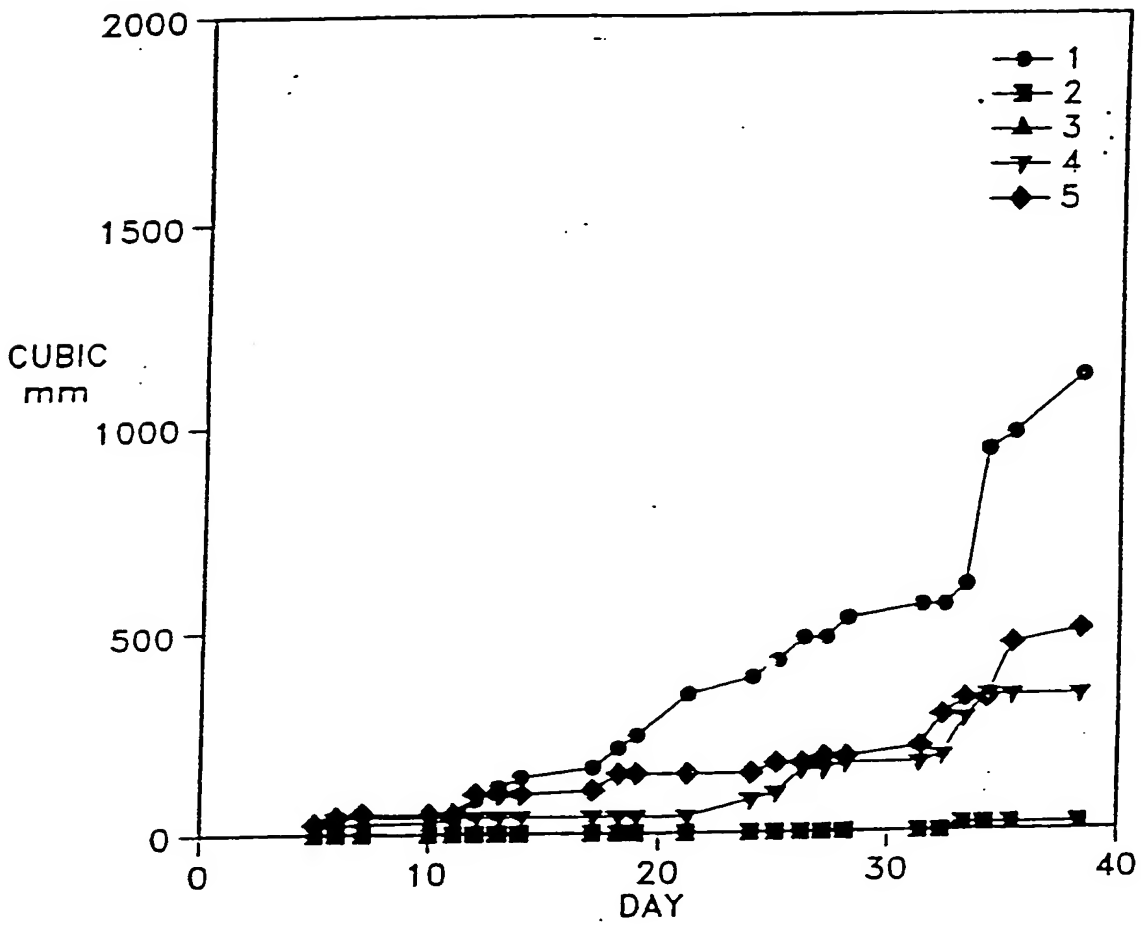

CONTROL
10% FBS+EC SUPPLEMENT
VEGF
RELATIVE CELL GROWTH
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
UNTREATED CELLS
COMPLETE MEDIA
VEGF
VEGF + MAb
VEGF + HEPARIN
VEGF + HEPARIN + MAb

FIGURE 14A

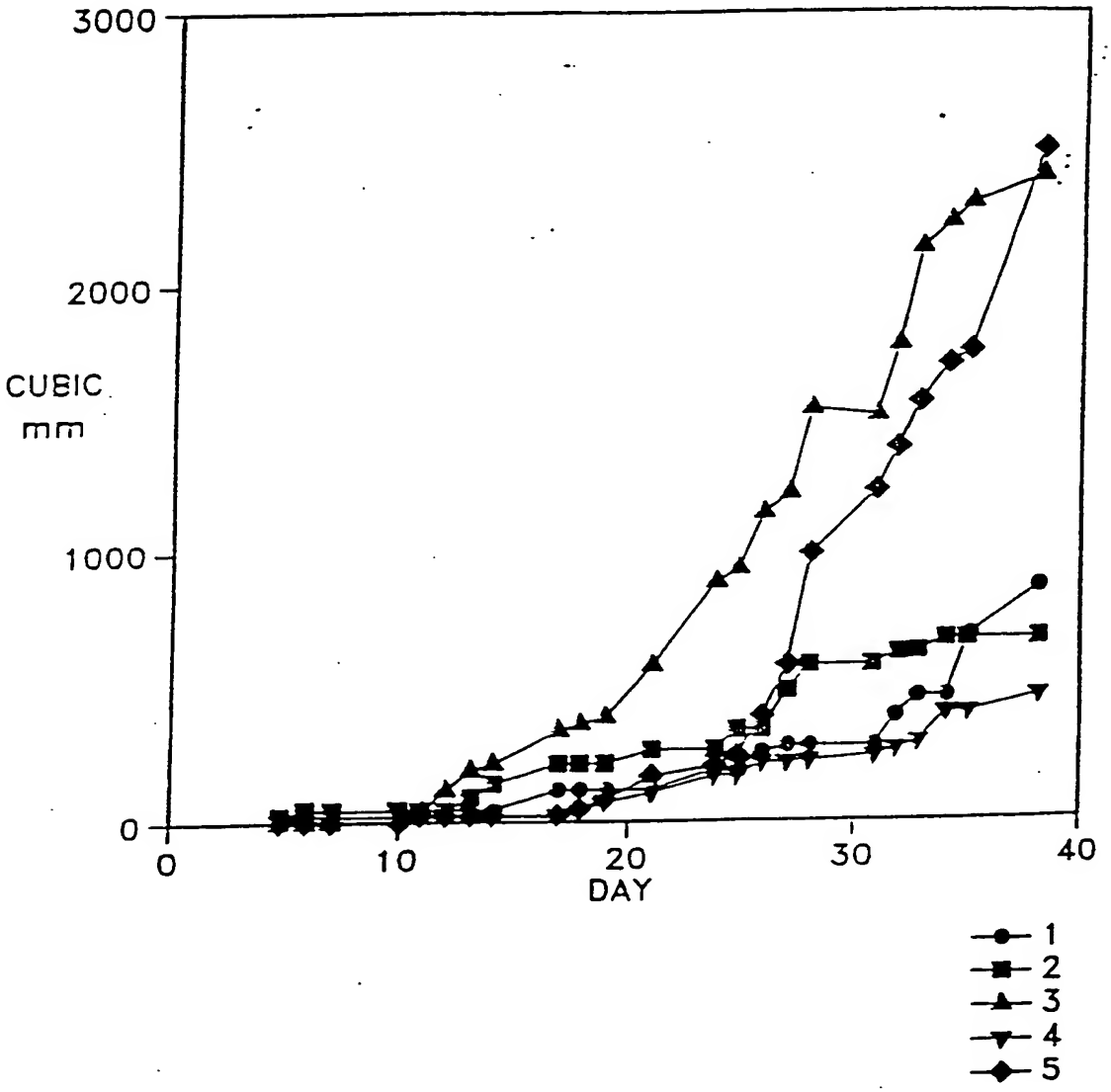
TREAMENT OF GLIOBLASTOMA
XENOGRAFTS WITH RAT anti-flk-1 MAb



STATISICAL ANALYSIS
flk-1 SLOPE = 16.09
p VALUE FOR flk-1 VERSUS flk-2 TUMOR SIZE = 0.0001

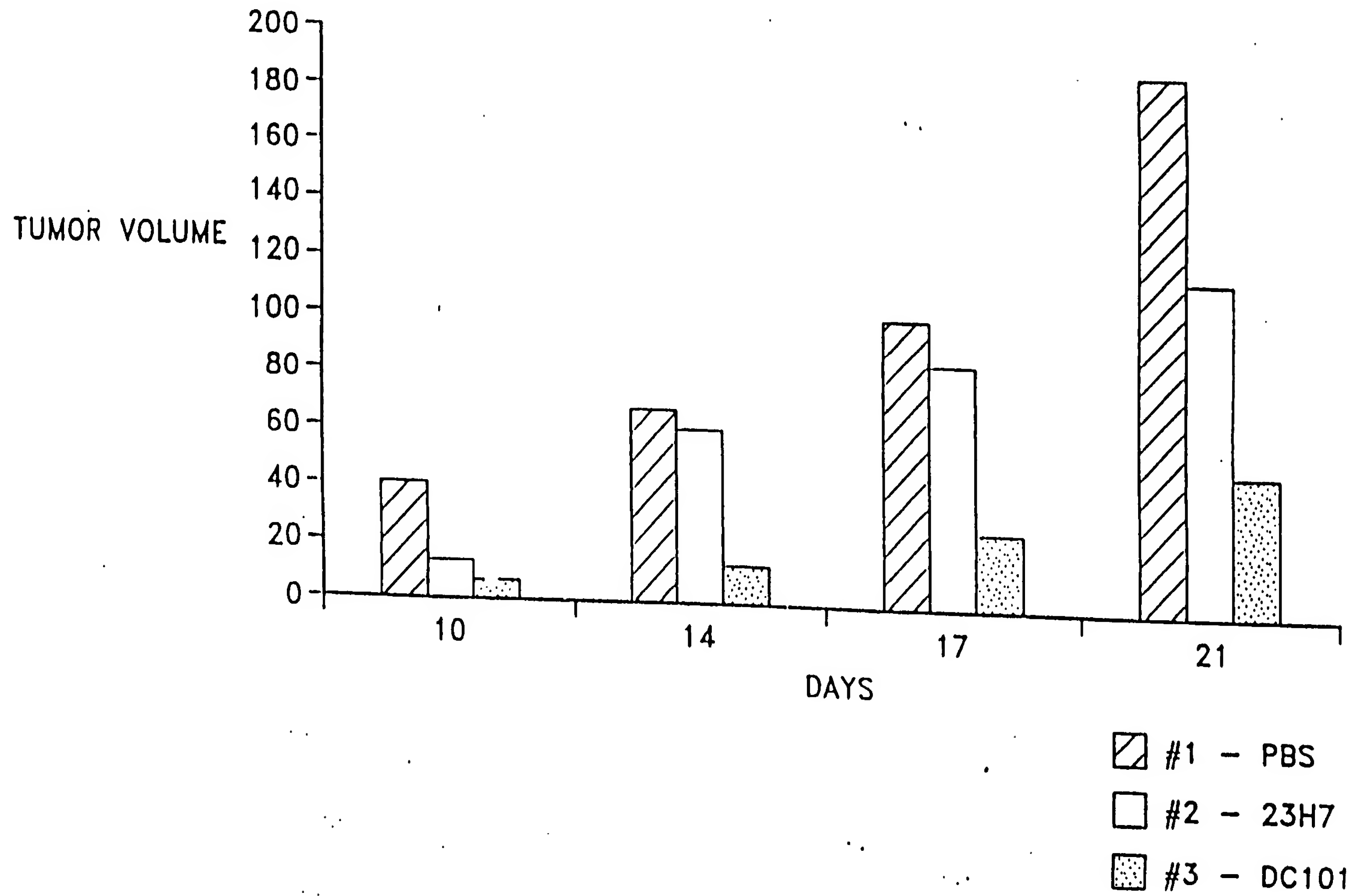
FIGURE 14B

TREAMENT OF GLIOBLASTOMA
XENOGRAFTS WITH RAT anti-flk-2 MAb



STATISICAL ANALYSIS
flk-2 SLOPE = 37.39
p VALUE FOR flk-1 VERSUS flk-2 TUMOR SIZE = 0.0001

FIGURE 15



TUMOR VOLUME
200
180
160
140
120
100
80
60
40
20
0
10
14
17
21
DAYS
#1 – PBS
#2 – 23H7
#3 – DC101

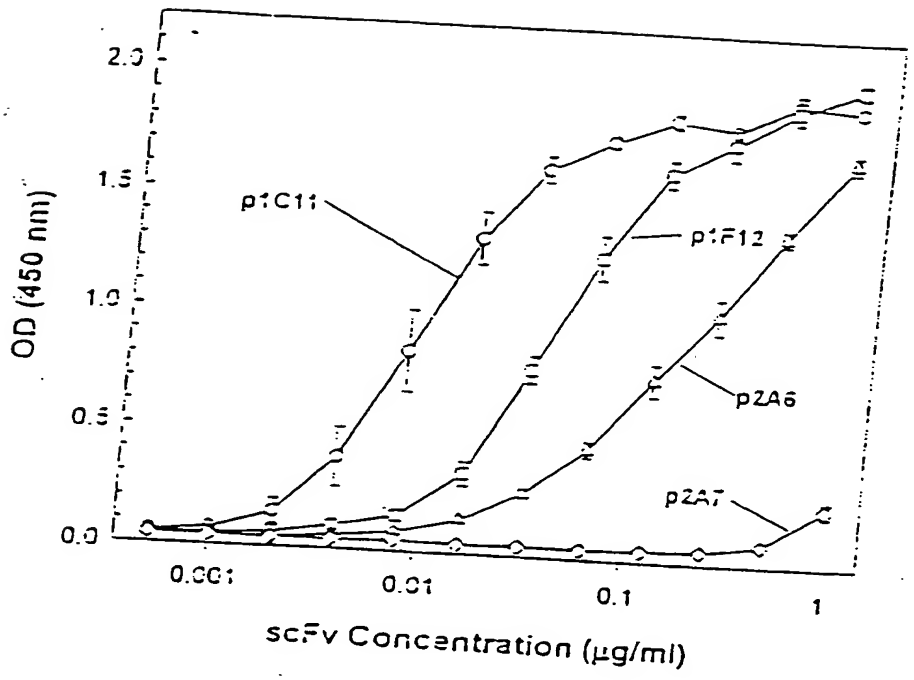

2.0
1.5
1.0
0.5
0.0
OD (450 nm)
p1C11
p1F12
pZA6
pZA7
0.001
0.01
0.1
1
scFv Concentration (µg/ml)

FIGURE 16

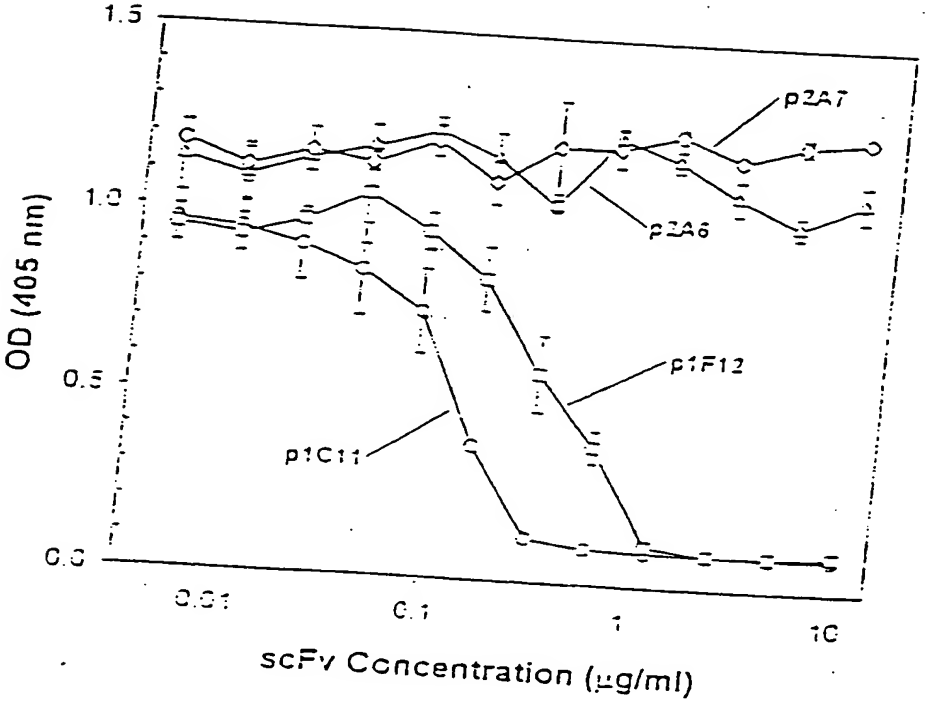

1.5
1.0
0.5
0.0
OD (405 nm)
p2A7
p2A6
p1F12
p1C11
0.01
0.1
1
10
scFv Concentration (µg/ml)

FIGURE 17

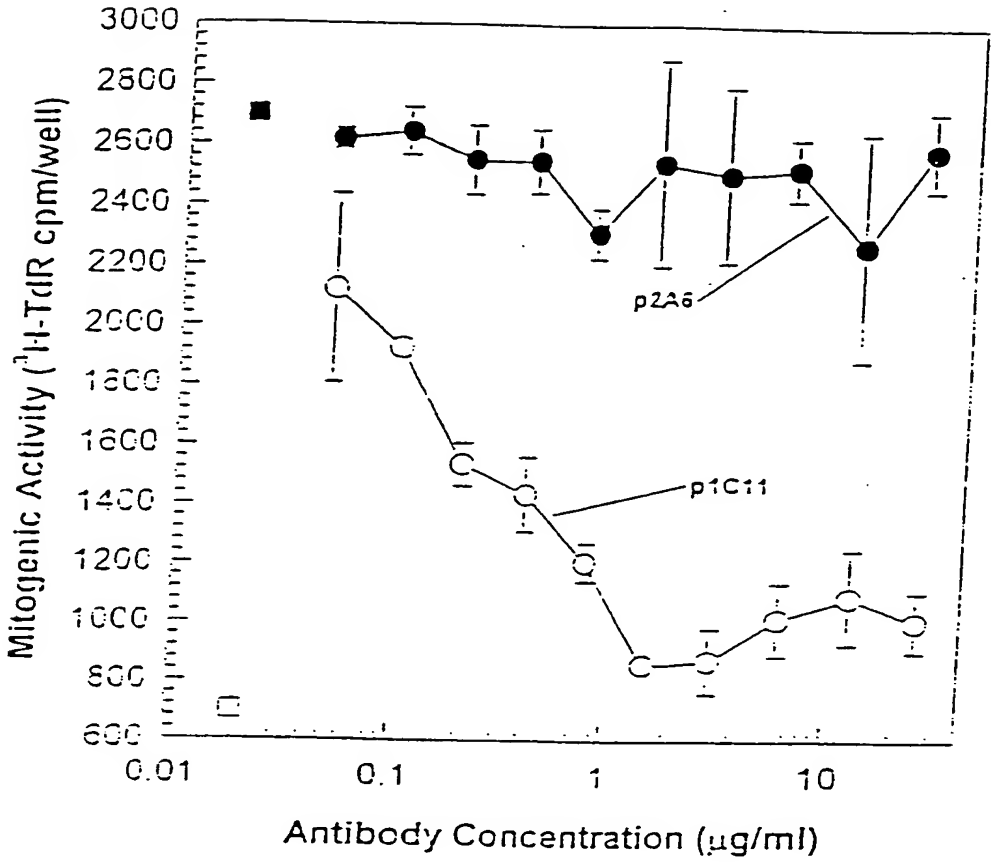

3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
Mitogenic Activity (^{3}H-TdR cpm/well)
p2A6
p1C11
0.01
0.1
1
10
Antibody Concentration (µg/ml)

FIGURE 18

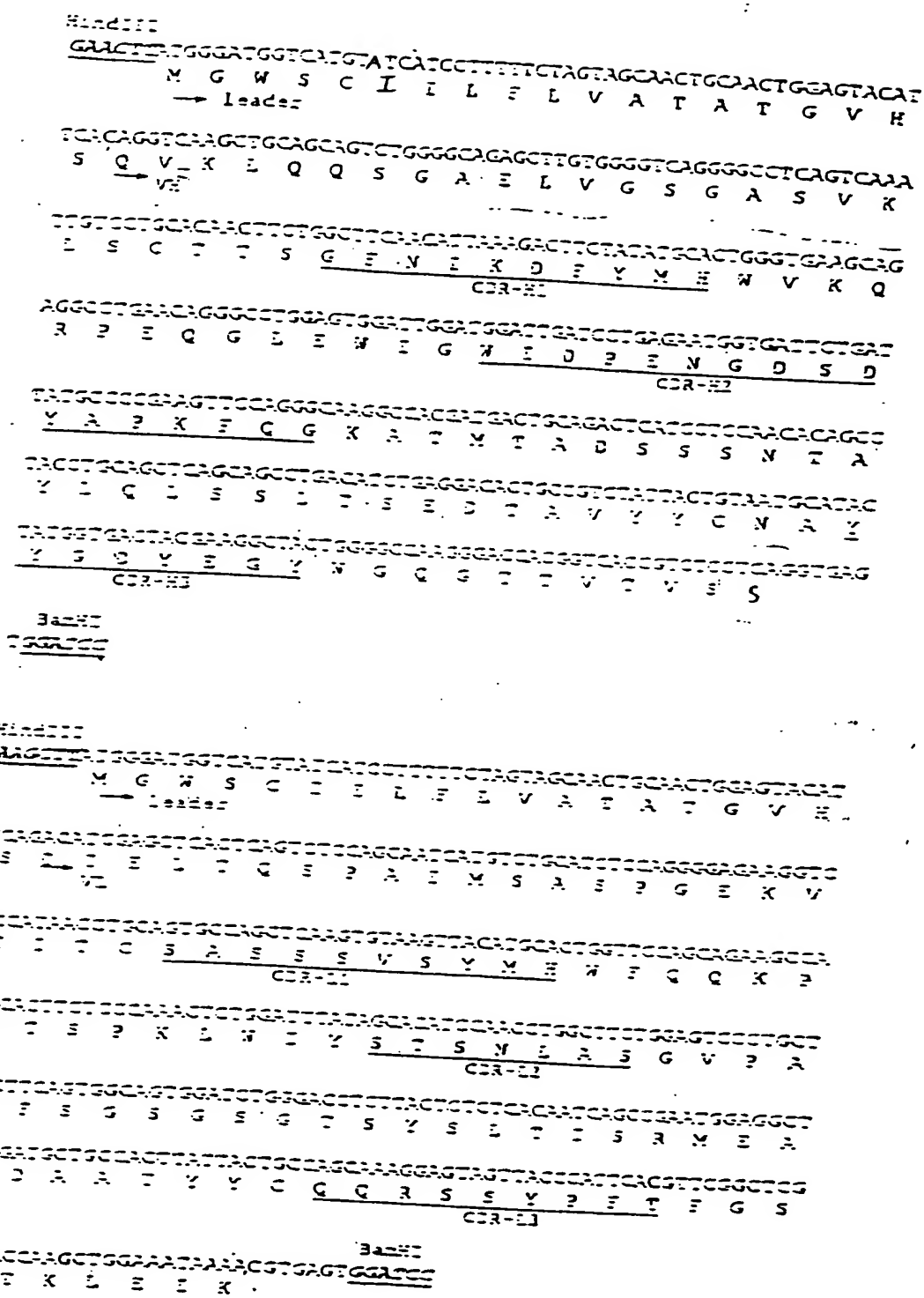

HindIII
M G W S C I I L F L V A T A T G V H
leader
S Q V K L Q Q S G A E L V G S G A S V K
VH
L S C T T S G F N I K D T Y M H W V K Q
CDR-H1
R P E Q G L E W I G R I D P A N G D S D
CDR-H2
Y A P K F Q G K A T M T A D S S S N T A
Y L Q L S S L T S E D T A V Y Y C N A Y
Y G D Y E G Y F N G Q G T T V T V S S
CDR-H3
BacHI
HindIII
M G W S C I I L F L V A T A T G V H
leader
S L T E L T Q S P A I M S A S P G E K V
VL
T I T C S A S S S V S Y M H W F Q Q K P
CDR-L1
G T S P K L W I Y S T S N L A S G V P A
CDR-L2
R F S G S G S G T S Y S L T I S R M E A
E D A A T Y Y C Q Q R S S Y P F T F G S
CDR-L3
BacHI
G T K L E I K

FIGURE 19

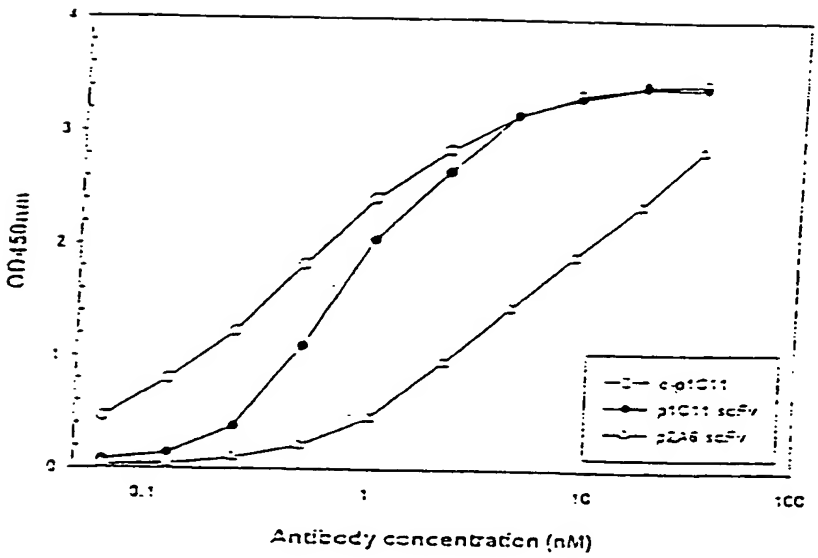

OD450nm
0
1
2
3
4
0.1
1
10
100
Antibody concentration (nM)
c-p1C11
p1C11 scFv
p2A6 scFv

FIGURE 20

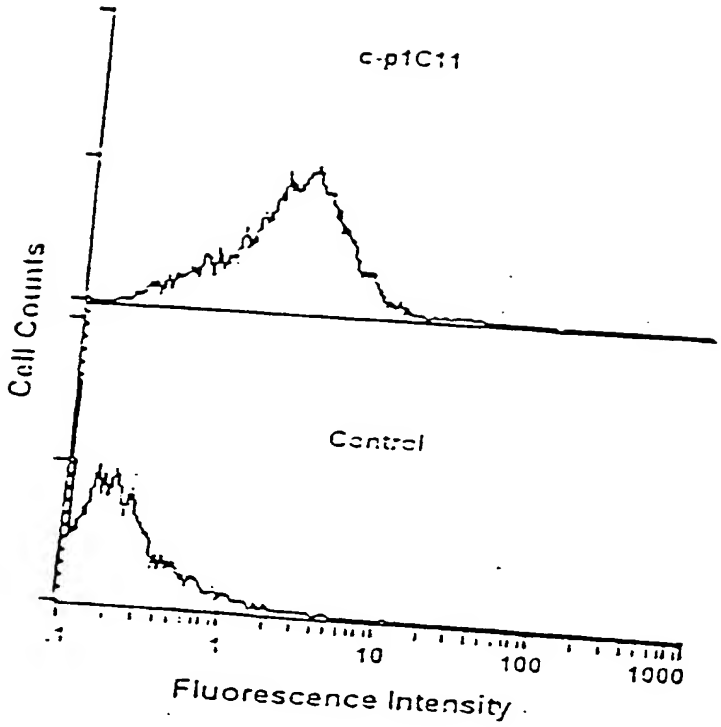

c-p1C11
Cell Counts
Control
1
10
100
1000
Fluorescence Intensity

FIGURE 21

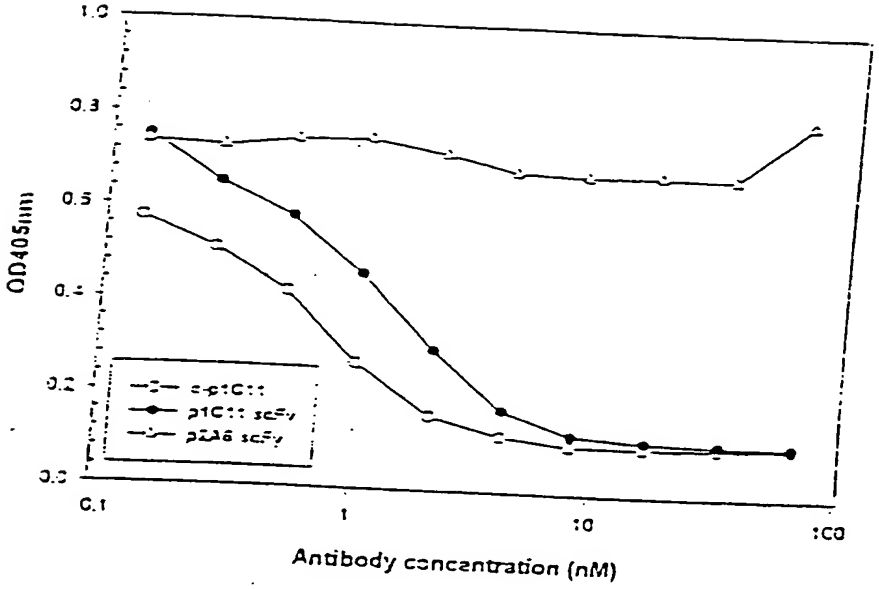

1.0
0.8
0.5
0.2
0.0
OD405nm
c-p1C11
p1C11 scFv
p2A6 scFv
0.1
1
10
100
Antibody concentration (nM)

FIGURE 22

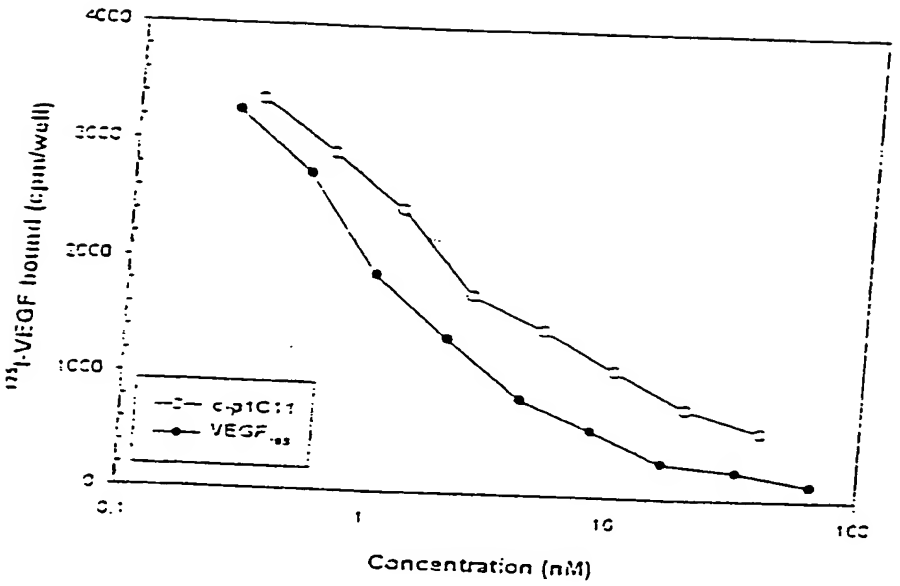

4000
3000
2000
1000
0
0.1
1
10
100
^{125}I-VEGF bound (cpm/well)
c-p1C11
VEGF$_{165}$
Concentration (nM)

FIGURE 23

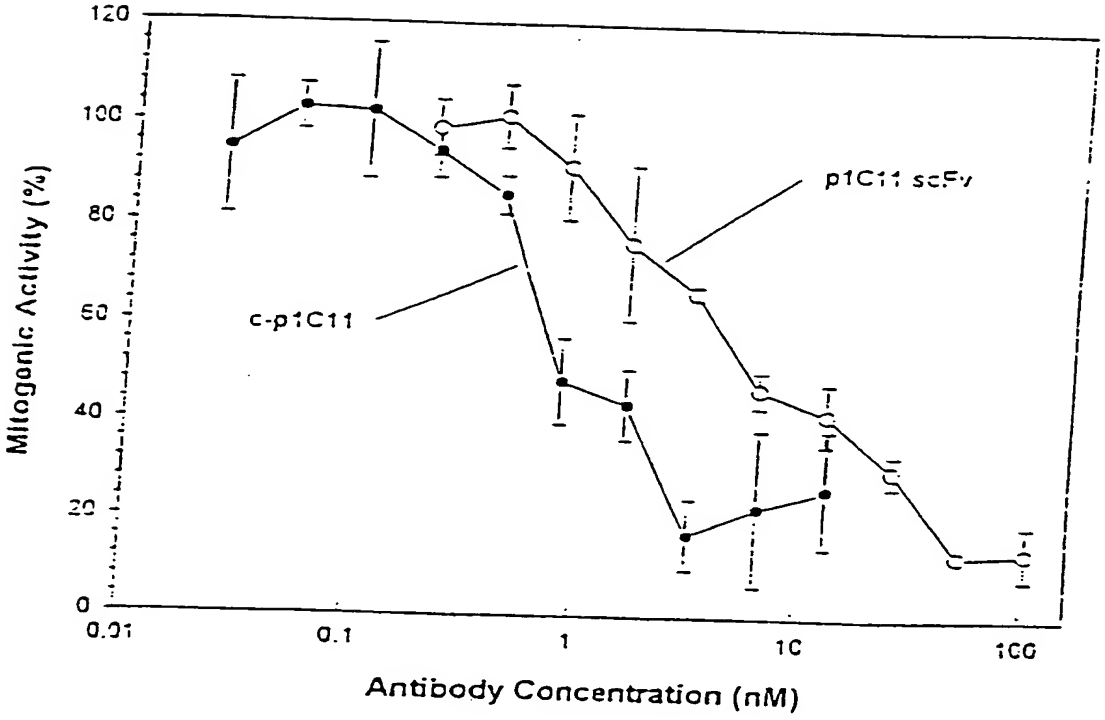

120
100
80
60
40
20
0
Mitogenic Activity (%)
0.01
0.1
1
10
100
Antibody Concentration (nM)
p1C11 scFv
c-p1C11

FIGURE 24